



## **TASTER RESPONSES TO ASTRINGENCY IN RED WINES**

**ENRICO PIGNONE**

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**VITICULTURE AND ENOLOGY DOUBLE - DEGREE**

Advisor: MANUEL José de Carvalho Pimenta MALFEITO FERREIRA  
Assistant: MARIANA da Silva Gomes MOTA

**Jury:**

President: Jorge Manuel Rodrigues Ricardo da Silva (Phd), Full Professor, at Instituto Superior de Agronomia, Universidade de Lisboa.

Members: Manuel José de Carvalho Pimenta Malfeito Ferreira (Phd), Assistant Professor, with habilitation at Instituto Superior de Agronomia, Universidade de Lisboa;  
Sofia Cristina Gomes Catarino (Phd), Invited Assistant Professor at Instituto Superior de Agronomia, Universidade de Lisboa.

*“Live, also drink, eat well, is think and  
weigh the qualities and separate  
the beautiful from the ugly,  
the good from the bad,  
the true from the false”*

*Luigi Veronelli*

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*This goal is for my family!*

## Abstract

The present work aimed to evaluate the responses of a panel tasters to astringency in red wines. The panel was trained on the tastes of the mouth in relation to the acidity, sweetness, bitterness and in particular to astringency.

Twenty-one subjects were selected through identification of basic tastes and binary taste mixtures. Tasters were introduced first to the theory of astringency and extensively trained to differentiate astringency from bitterness and sourness using 3.0 g/L tannic acid, 0.25 g/L caffeine and 4 g/L tartaric acid to first in water then in white wine. Tasters were segmented according to gender, 6-n-propylthiouracil (PROP) status, saliva flow and Vinotype.

The tests determined the detection and recognition thresholds of commercial seed grape tannin (CT) in white wine (0.95 g/L and 1.21 g/L, respectively). These thresholds were not related with response to PROP or saliva flow.

Astringency and persistency of 5 commercial red wines showed a positive correlation ( $r=0.71$ ,  $p<0.0001$ ). One sample was used as control wine (WC) composed by Arinto base wine (ISA) spiked with supra-threshold concentrations of seeds commercial tannins (1.5 g/L) and tartaric acid (1.2 g/L). Significant differences were observed between the most and least astringent/persistent wines. These mouthfeel properties were not related with wine preference.

When comparing the evaluations of astringency and persistency of all wines, PROP status and Vinotype segments did not produce different responses. On the contrary, males and higher salivators gave higher ratings than females or low salivators, respectively. However, this gender or saliva flow based differences were dependent on the tasted wines.

The overall results showed that astringency is a mouthfeel sensation difficult to establish because of the different individual responses. In addition, wine preference was similar across a wide range of astringency indicates that this sensation could play a small part in wine liking when considering alone.

**Key-words:** Astringency, Sensorial Analysis, Taste Phenotype, Sensory Threshold, LMS (labeled magnitude scale).

## Resumo

O presente trabalho teve como objetivo avaliar as respostas de um painel de provadores à adstringência em vinhos tintos. O painel foi treinado sobre os gostos da boca em relação à acidez, doçura, amargor e em particular à adstringência.

Vinte e um indivíduos foram escolhidos, as prova incluíam gostos básico, misturas binárias e classificação de intensidade de sabor. Familiarizado em cada dez sessão de treino de uma hora, incluindo: introdução à teoria de adstringência e extensivamente treinados para diferenciar de adstringência amargura e acidez com 3,0 g/L tânico ácido, 0,25 g/L cafeína e 4 g/L ácido tartárico para localizar os diferentes estímulos (ácido, adstringentes doce, azedo e) em misturas binárias na água primeiro e depois no vinho branco. Os degustadores foram segmentados de acordo com o sexo, status de 6-n-propiltiouracil (PROP), fluxo salivar e Vinotype.

Os testes determinaram os limiares de detecção e reconhecimento de taninos comerciais de grainha de uva (CT) em vinho branco (0,95 g / L e 1,21 g / L, respectivamente). Esses limiares não foram relacionados com a resposta ao PROP ou fluxo de saliva.

Astringência e persistência de 5 vinhos tintos comerciais apresentaram correlação positiva ( $r = 0,71$ ,  $p < 0,0001$ ). Uma amostra foi usada como vinho padrão (WC) Arinto (ISA), adicionado com concentrações supralimiais de taninos comerciais de grainha de uva (1,5 g / l) e de ácido tartárico (1,2 g / L). Observaram-se diferenças significativas entre os vinhos mais e menos adstringentes/persistentes. Estas propriedades na boca não estavam relacionadas com a preferência do vinho

Na comparação das avaliações de adstringência e persistência de todos os vinhos, o estado de PROP e os segmentos Vinotype não produziram respostas diferentes. Pelo contrário, os homens e os salivadores maior deram avaliações mais elevadas do que as mulheres ou salivadores menor, respectivamente. Estas diferenças de sexo ou de fluxo de saliva são os resultados dos vinhos provados.

Os resultados gerais mostraram que a adstringência é uma sensação difícil de determinar devido aos diferentes respostas individuais. Também, a preferência para o vinho foi semelhante dentro o diferente nível de adstringência mostrando que essa sensação só joga uma pequena parte no gosto do vinho quando considerado isolatamente.

**Palavras-chaves:** Adstringência, Análise Sensorial, PROP status, BET (best estimate threshold), LMS (labeled magnitude scale.)

## Resumo Alargado

O presente trabalho pretendeu avaliar a aceitação, percepção e preferência por adstringência no vinho tinto, de acordo com a segmentação de consumidores em idade, género, sensibilidade gustativa e nível de experiência de consumo de vinho.

De acordo com a definição da Sociedade Americana de Testes e Materiais, adstringência refere-se ao "complexo de sensações devido ao encolhimento, desenho ou rugas do epitélio como uma resultado da exposição a substâncias como o ex-alunos ou taninos "(ASTM, 2004). A adstringência pode ser considerada como um atributo negativo pelo consumidor se vinhos foram percebidos como dura e áspera, porque o equilíbrio gustativo é deslocada para o componente tânico, mas não é fácil, também para os técnicos do vinho, distinguir com toda a sensação do vinho tinto, em particular com a amargura.

No vinho, que contém uma grande quantidade de polifenóis, o sabor de amargura é desencadeada principalmente por flavan-3-ols e seus polímeros embora também seja capaz de ser provocada por alguns flavonóides hydroxycinnamates e benzoico derivados do ácido.

Não há um vocabulário comum para definir e descrever adstringência do vinho, por causa do diferenças culturais, percepção e prazer desta sensação. Este estudo foi realizado para restringir e investigar todas as variáveis e relação entre os sujeitos, que foram escolhidos, o compete do trabalho incluiu o sabor básico e as misturas binárias e sabor de classificação de intensidade. Familiarizado em cada dez sessão de formação de uma hora, incluindo: introdução à teoria de adstringência e extensivamente treinados para diferenciar adstringência de amargura e acidez com 3,0 g / L tânico ácido, 0,25 g / L cafeína e 4g / L ácido tartárico.

Eles foram convidados a identificados os diferentes estímulos (ácido, doce, azedo e adstringente) em misturas binárias de água em primeiro lugar, em seguida, no vinho branco. Painelistas estavam familiarizados classificação com adstringência, avaliando adstringência geral de concentrações diferentes: de 0,1 a 5 g / L de CT (tanino comercial) em uma escala de 9 pontos (ausente, muito fraco, fraco, fraco a moderado, moderado, moderado forte, forte, muito forte, muito forte), primeiro em água e depois em vinho branco. Em cada sessão de cinco amostras desconhecidas (10 ml) foram apresentados em uma ordem aleatória equilibrada à temperatura ambiente em copos em forma de tulipa negra codificados com números aleatórios de 3 dígitos.

Os avaliadores foram instruídos a pour toda a amostra na boca, mantê-lo para oito segundos, expectorar e avaliar a adstringência percebida geral usando uma escala de 9 pontos.

Os provadores esperaram durante 4 minutos antes de enxaguar com água deionizada por 10 segundos, duas vezes, e então esperou no menos trinta segundos antes da próxima amostra, cinco minutos para cada amostra. No início de cada sessão, os participantes provaram a seleção padrão de referência para a adstringência: 3 solução CT (0,1 muito fraco, 2,5 moderada, extremamente 5).

A formação tem permitido avaliar a BET (melhor estimativa avaliação) de todos os componentes, mostrando uma relação com o PROP. A utilização das escalas de medição suprathreshold pode aumentar a probabilidade de detectar diferenças nestes sensações induzidas por vinhos tintos em indivíduos de diferentes grupos provador PROP, reavaliados pelo tendo os participantes avaliar a amargura PROP três soluções (0,032, 0,32, e 3,2 mm) que foram provadas por ordem de concentração. Os participantes receberam 20 ml de solução e instruído para mover a amostra de lado a lado na boca durante 5-10 s e depois para expectorar. Depois de esperar por 10-15 s, eles usaram o LMS para avaliar a amargura da amostra. Posteriormente, foi registrada a quantidade de saliva para cada um dos provadores, a fim de comparar com o parâmetro os resultados obtidos anteriormente

Em fim usando o LMS (labeled magnitude scale), foram convidados para avaliar o nível de adstringência, persistência e preferência na prova de seis vinhos comerciais de cinco regiões diferentes de Portugal, expressões de diferentes terroirs e estilos de vinho.

Após as provas, foram realizados testes estatísticos aos dados, nomeadamente análises de variância a cada fator para cada concentração de CT (tanino comercial). Os resultados foram tratados de acordo com uma análise à sua variância.

Relativamente ao Gender e saliva flow houve uma directa significatividade intra os non-taster, taster e supertasters, de acordo com uma melhor sensibilidade em função do fluxo de saliva. O BET mostrou diferenças significativas na concentração de 0,4 mg/L CT (tanino comercial).

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## **LIST OF ABBREVIATION**

PROP - 6-n-propylthiouracil

HF - High flow rate

LH - Low flow rate

CT - Commercial tannins

ISA - Instituto Superior de Agronomia

gLMS - general Labeled Magnitude Scale

gDOL- generalized Degree of Liking

W1- Piteira DOC Reserva 2012

W2- Marquês de Marialva DOC Reserva 2010

W3- Quinta das Bageiras Colheita Tinto 2012

W4- Casas Altas DOC Reserva 2011

W5- Aguião IGT 2014

WC- Wine control, Arinto from ISA

TDS- Temporal Dominance of Sensation

# 1. Introduction

The wine has always had the capacity to “release the tongue” and make the others listen in a social context. It's creation and consumption is combined by speeches, culture, exchange of ideas, man's work and pleasure of sharing that has been passed since the Symposium Greek time through the music, dance, food, eros and literature. Source of insight and inspiration, the wine is the area's identity, demarcation of the border through a micro and macro-zone as to identify the vineyard itself, the climate, the geographical position, the "terroir" (Rota and Stefi, 2012). As well as food, it has transformed the natural necessity to eat without being hungry and drink without being thirsty, because it comes from reflection on the pleasures of the table and by the desire to prolong it. (Brillat-Savarin, 1960), evolving into a real art: the art of degustation. Specifically Peynaud (1981) in the “Taste of wine”: “There is much difference between drinking and tasting. Good wines, great wines are not drinks that you ingest: you must taste. They are never taken in big gulps like a refreshing drink, to capture the only tactile feeling of the liquid that cools the throat. [...] The act of drinking wine and its meaning are not those of the instinctive drinker, the technique of drinking is very different: you learn. [...] [...] Result of many efforts, science and patience, good wine, great wine, deserves a commitment from the one who wants to know, that comes to him, taster, the message of those who have worked for the pleasure of others. The tasting is used to decipher this message and to codify the taste sensations. To drink just physical pleasure, to taste it also takes intelligence and competence ”.

## 1.1 Sensory analysis

The Sensory analysis differs from tasting because include the strict control of assessing, with an addition of three fundamental parameters: the response of a panel taste (number of evaluation), assessment of specific test, (modalities of evaluations) and use of statistical analyses (monitoring of evaluations) (Sangiorgi, 2011). Was born about fifty years ago (when the US consultancy Arthur D. Little proposes to agribusinesses the first formal method to describe the taste of food) to analyze the sensations produced by food and drink, finding broad consensus and development. (Cavaliere, 2011). In general the role of the panel is to give an expectation of consumers in accordance with market economy principles, develop new products and improve those that already exist, reducing costs and checking the quality, also fundamental for the storage stability. Different panels can be formed by people with different levels of knowledge and experience of the product to be tested. Several testing is used to determine if different winemaking processing techniques or operations have a sensory impact. There are four main types of tests:

- Triangle; tasters assess all three samples then pick the sample which is different from the other two, or the odd one.
- Duo-trio; tasters assess the reference (Ref), then the two test sample (A, B). Tasters are asked to indicate which test sample is the same as the reference.

- Paired comparison; tasters are asked to identify which sample is higher in an attribute (e.g., identify which sample is sweeter).
- Same/Different; tasters assess both samples and indicate whether they think samples are the same or are different.
- Paired preference; tasters assess both samples and indicate which one they prefer. A choice must be made; the taster can't say they prefer neither.

The principles of the tasting are based on memory: it's easier to recognize and identify only what has already been heard and recorded, even better if an odor or taste is associated with a name which should facilitate the memory. The professional taster also requires an agile mind, to find in a few moments, the personal archive traits taste-olfactory present in the mouth. Simultaneously it is necessary a vocabulary acquisition of tasting, terminologies regulated for the purpose of mutual understanding of the experts and the sharing of subjective sensory experience: it's easy to understand why most of the efforts of this discipline will be addressed to the encoding of a language professional. Subjectivity determines large differences between the tasters' interpreting and the sensations produced by the same stimulus, for the use of descriptors and the variability associated with different circumstances. Nowadays, the development of new analytical equipment such as 'electronic noses' provides much better results in identifying odor patterns (Buratti et al., 2004; Martí et al., 2005), as well as the saliva precipitation index (SPI) and particle size distribution to evaluate the astringency intensity of tannin by the means of SDS-PAGE electrophoresis and dynamic light scattering methods, respectively (Pascal et al., 2007; Rinaldi et al., 2010). These kinds of equipment could prove more reliable to make the chemical profile of wines but will probably not be able to replace human capacity to actually appreciate the other dimensions that wine brings and that have not been, so far, translated into a chemical formula: pleasure, satisfaction, a whole range of positive emotions (Coste, 2015). Ubigli (2004) observed "the sensory signal, in the strictest sense, is complemented by a multitude of other information of a hedonistic and emotional type" and perception is not just about a physiological reaction, but an "activity that involves knowledge and reflection".

#### **1.1.1. From the numbers to the emotions**

In the forties with the school of Davis, the analytical language has developed in support of a large-scale production of the wine industry. In this context, new wine method evaluation was proposed, advancing a quantitative and universal conventional assessment: numerical measure accompanied by a referential lexicon (Perullo, 2009). Journalists and specialists in the field, have introduced several wine competitions intent to communicate and promote international wines in order to suggest and direct the consumer towards the best buy. The tendency of this approach has



created a specific “style” of wine, favoring “easy” international commercial wines in opposition to the “difficult” European classic wines (Loureiro et al., 2016), usually hesitant, not very clear nose and rough taste. These types of wines can be expressed more clearly with the time for “breathing” in the glass or in the open bottle, showing more fragrant, rich in flavor and more harmonious (Rizzari, 2015). New propose to design and evaluate a different tasting sheet including emotional reactions, in order to develop and improve the traditional wine tasting sheets. This approach was introduced by Ferrarini et al. (2010), Rive and Deneulin (2014), and recently merging with the inclusion of a focus group (Coste, 2015).

## **1.2. Astringency**

The term astringency derives from the Latin verb *a-strigo* (*ad-stringo*), *strinxi*, *strictum*, *a-stringere* that means tightly bind, strongly join. It refers to the propensity of vegetable tannins to complex with macromolecules, such as proteins and polysaccharides, and alkaloids. The importance of tannin-protein interactions in ecological systems is illustrated by the reduced palatability of high tannin forages to herbivores (Mehansho et al., 1987). The name of “tannins”, originating from “tanning” of leather, has been used over decades for their capability of binding with proteins (Gawel, 1998), whereas it is often described in sensory terms as a roughing, drying or puckering sensation in the mouth (ASTM, 2004). Bate-Smith (1954) first speculated that astringent sensations were caused by the increase in friction between the mucosal surfaces which resulted from the reduction in lubrication as salivary proteins were bound by astringent compounds. Often confused with bitterness, astringency was also defined as the “dry-mouth” feeling thought to be produced by the interaction of polyphenols with the proteins of the mouth (Singleton and Esau, 1969).

Astringency then can be defined as a tactile sensation, because: (i) it is perceived on non gustatory surfaces such as on soft palate, gingives, lips, (ii) does not show adaptation but also (iii) increases upon repeated ingestion. (Rinaldi et al. 2012).

### **1.2.1. Tannins**

The flavonoids are a group of compounds based on the polyphenolic flavan skeleton, variously substituted with hidroxyl, methyl, galloyl, glucosyl and acyl moieties. In addition to absorb both UV and visible light, including pigments such as aurones, chalcones, and anthocyanins, act as attractants for pollination and seed dispersal (Koes et al. 1994, Shirley 1996, Smith and Markham 1998). The biosynthetic sequence leading to flavonoids begins at the chalcone synthase reaction where *p*-coumaroyl-CoA combines with three malonyl-CoAs to give a chalcone and  $\text{Co}_2$  (Douglas 2006). Tannins responsible for wine astringency, consist mostly of flavan-3-ol polymers that are commonly referred to as proanthocyanidins or condensed tannins (De Freitas & Mateus, 2001). They are biosynthesized via a complicated path beginning with phenylalanine (less often tyrosine) and then polymerize proanthocyanidins (Ali, K. et al., 2010).

Tannin is a generic term gathering 3 groups of structurally different molecules: phlorotannins, condensed tannins, and hydrolysable tannins. Individual phlorotannins are composed of two or more phloroglucinol (Fig. 1.1. a) units that are attached to each other via C-C or CO-C bonds, thus yielding oligomers such as the tetrameric phlorotannin (Fig. 1.1. b). Further structural variations may include additional OH-groups in the molecules or additional bonds between the monomers. (Rinaldi A, et al. 2012).

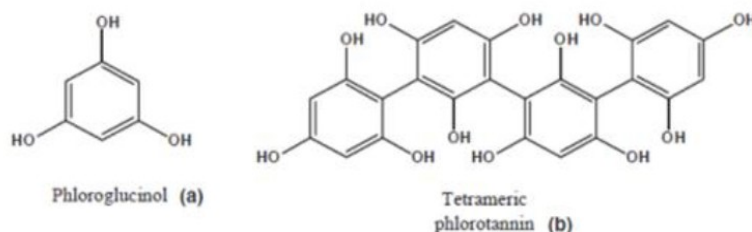


Figure 1.1. Structure of phloroglucinol (a), the phlorotannin unit, and a tetrameric phlorotannin (B) consisting of four phloroglucinol units.

Condensed tannins represent a large majority of tannins present in grapes and wines. They are polymers with more than three units composed of terminal and extension subunits analogous to the flavan-3-ols catechin, epicatechin, epicatechin-gallate, and epigallocatechin (Fig. 1.2.). In a hot and acid medium the interflavanic bond breaks down releasing an unstable carbocation producing, eventually, an anthocyanin. For this reason flavan-3-ol polymers are also called proanthocyanidin (Bate-Smith, 1975).

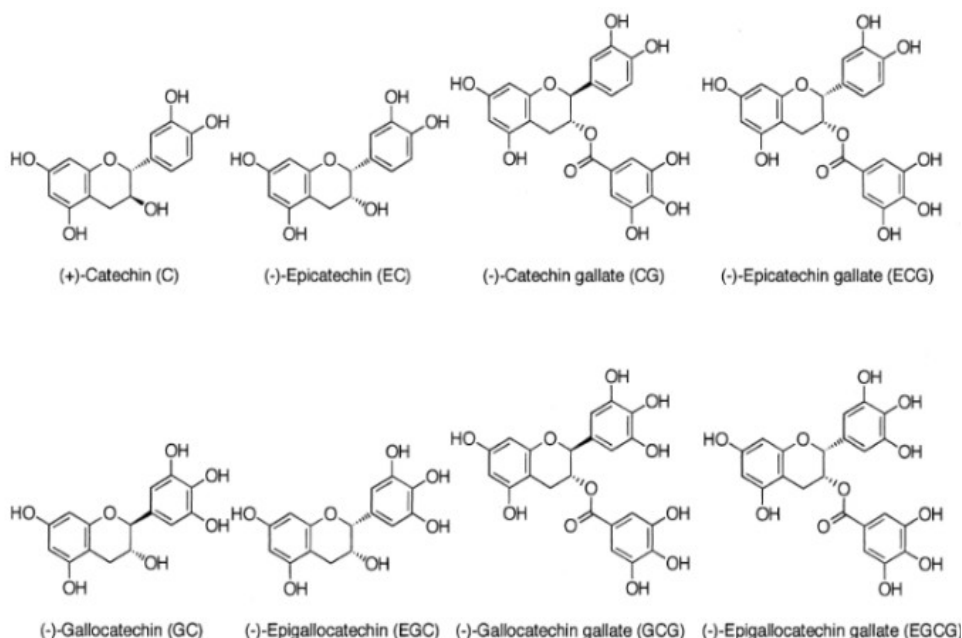


Figure 1.2. Common monomeric units of condensed tannins

Condensed tannins, whose composition is listed in tab. 1, are extracted from grape seeds, skins, and stems during the winemaking process (Ribéreau-Gayon, 1974)

Table 1. Composition of skin, seeds and stems tannins (from Souquet et al., 2000).

TANNINS		
SKINS	SEEDS	STEMS
(+) catechin	(+) catechin	(+) catechin
(-) epicatechin	(-) epicatechin	(-) epicatechin
(-)epicatechin-3-O-gallate	(-)epicatechin-3-O-gallate	(-)epicatechin-3-O-gallate
(-) epigallocatechin		(-) epigallocatechin
mDP* = 30	mDP = 10	mDP = 9
Galloylation = 3-10%	Galloylation = 20-40%	Galloylation = 15-20%
High mDP	Oligomers=60%	High mDP

\* mDP= mean degree of polymerization

The tannins in the grape vary in size from dimers and trimers up to oligomers with more than 30 subunits. As showed in the table 1, skin tannins differ from seed tannins for the average size (expressed as their mean degree of polimerization: mDP), larger than seed tannins; skin and stems tannins contain epigallocatechin subunits whereas is absent in the seed. Among the skin flavanols, polymers form are more represent than oligomers, while the monomeric catechins are under-represented (Mateus et al. 2001).

A class of tannin present in wine derived from the use of wood barrels, wood chips or oenological tannins (Ribéreau-Gayon 1972, Saucier et al., 2006) is represented by hydrolyzable tannins. Wine storage with winewoods had a sweetening effect and in parallel decreased the astringency and bitterness sensation (Chira et al. 2013).

Tannic acid, commonly used as a reference compound for astringency in sensory studies, is a mixture of gallotannins with impurities, such as gallic acid and various gallic acid derivatives (Salminen and Karonen, 2011).

### 1.3. Mechanism of perception

Astringency intensity in wine is correlated to the sum of binding affinity for each tannin molecule, which is determined by tannin concentration and their chemical structure characteristics. Proanthocyanidins are mostly responsible for the astringency, bitterness and structure of wines (Gawel, 1998), while they also play an important role in red wine aging due to their high reactivity: polymerization, condensation with anthocyanins and oxidation reactions (Ricardo-da-Silva et al., 1991). Astringency intensity in wine showed a strong positive relationship with tannins concentration (Robichaud et al., 1990). The study of Peleg et al. (1999) demonstrated that astringency intensity of pure tannins increased significantly, but when the polymerization degree of condensed tannin is too high, it will induce self-precipitation. The esterification of proanthocyanidins with addition of gallic acid plays a significant role in the protein binding

capability (Soares et al. 2007). Flavan-3-ol monomers containing a galloyl ring in the molecule led to an increase of the friction coefficient in the oral mimetic surface in the study of Rossetti et al. (2009). As show figure 1.3, the possible astringency mechanisms include: a) the interaction between stimuli and proteins with formation of soluble complexes and insoluble sediment, b) (i) lack of saliva film and eventual bind to the receptors exposed, (ii) insoluble stimuli-protein complexes trigger astringency sensation via increasing friction and (iii) interaction with oral cavity membrane.

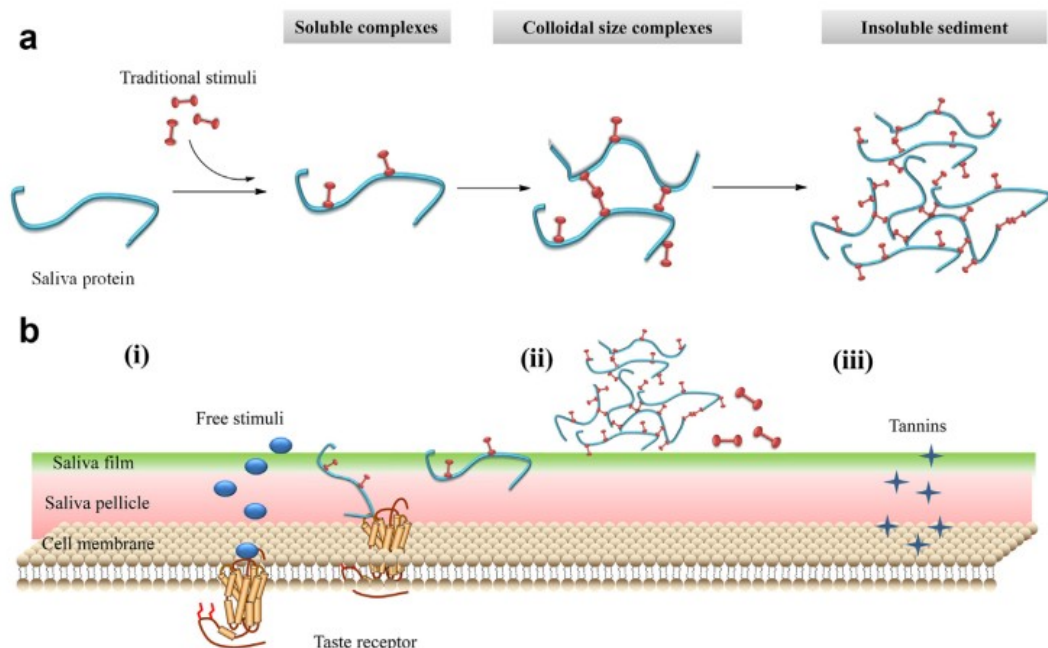


Figure 1.3 Mechanism of astringency perception (from Ma, W., et al., 2014)

This process is believed to be due to the astringent sensation experienced when salivary proteins are precipitated by tannins and as a result lose their ability to lubricate the epithelial membranes of the mouth. (Rinaldi et al, 2010). The sensation of puckering astringency may be elicited by the coactivation of trigeminal chemosensors and mechanosensors, whereas velvety astringents might exclusively activate trigeminal mechanosensation (Schöbel et al. 2014).

The perception of astringency is basically a dynamic process that continuously changes and evolves, takes many seconds (15 s) to develop fully. The TDS (Temporal dominance of sensations) sensory studies on wine, stated that after the sweet, sour and bitter tastes, at about 35 sec after expectoration or ingestion, astringency takes place (Pessina et al., 2004).

#### **1.4 Aims of the study**

This research was based on training and tasting sessions of a selected panel to evaluate their sensory and preference responses to several red wines. The panel was subjected to segmentation according to gender, PROP status, saliva flow and Vinotype. In particular the objectives of the study were:

1. To determine the BET (best estimate threshold) of commercial grape seed tannins in white wine.
2. To evaluate the sensory responses to astringency according to taster segmentations.
3. To evaluate the preference for red wines with different astringency levels.

## 2. Material and Methods

### 2.1. Taster Selection

The tasting panel was mainly selected among the students of the Master of Viticulture and Oenology of the Instituto Superior de Agronomia (2015/2016). First and second-year students were the main targets for the work. The selection has been conducted in order to find the subjects with the best knowledge and sensitivity about the main descriptors of the mouthfeel: acidity, sweetness, bitterness and astringency with the purpose to have a group of people trustable in the results and trained to perceive differences. During training sessions, white and red wines from the ISA (see Annex 10) were used in order to prepare different solutions with several reagents.

The guideline was to find out subjects that consume usually wine at least one for the week and able to distinguish the samples submitted. They were prepared, highlighting the main mouthfeel perception. The work started contacting students, males and females, from the ISA without any age limit, smokers and non-smokers.

#### 2.1.1. Training session

The training session was made with the basic tastes. The first tasting was with aqueous solutions with the reagents mentioned in table 2.1.

Table 2.1. Reagents and concentrations of aqueous solutions for the first training sessions (from Ceciliani 2017)

Taste/Sensations	Concentration	Reagent
Sweet	10 g/L	Sucrose
Sour	1 g/L	Tartaric acid
Sour	1g/L	Malic acid
Sour	1g/L	Lactic acid
Bitter	0.0108 g/L	Quinine Sulphate
Astringency	0.8 g/L	Aluminium Sulphate
Astringency	0.5 g/L	Grape Skin Tannin
Hot	10 % (v/v)	Ethanol

The solutions were served in transparent ISO tasting glasses from Schott Zwiesel (Zwiesel, Germany). Participants were given a questionnaire sheet and the tasting had 3 parts. Tasters were asked to detect and recognize the feeling or sensation in the sample. In the first part tasters were given the sucrose, the tartaric, the quinine sulphate, the aluminium sulphate and the ethanol solutions; they tasted and answered in the sheet. In the second part, participants were given two glasses each with a solution of malic acid and lactic acid, respectively, to express the difference between the samples. Lastly, in the third part students were given two glasses with the Aluminium

Sulphate solution and Skin Tannin solution, respectively, for the participants to feel the different astringency. We asked the participants to drink water between every sample they tasted and to spit out the sample.

Sucrose was purchased from Sigma (St. Louis, USA), L(+) – Tartaric Acid was purchased from Panreac (Barcelona, Spain). Quinine Sulphate was purchased from Acofarma (Barcelona, Spain), Aluminiumsulphat-18-hydrat was purchased from Riedel-de Haën (Seelze, Germany), Ethanol 96% was purchased from Aga (Lisbon, Portugal), L(-) Malic Acid was purchased from Sigma (St. Louis, USA), DL –Latic Acid was purchased from Sigma (St. Louis, USA), Skin Tannin (Tanin Vr Grape) was purchased from Laffort (Bordeaux, France).

After one week, the second tasting was done with a mixture of the tastes and sensations used in the first tasting, using more than one sensation/taste per glass. In the last 3 glasses we used different wine additives with mouthfeel properties to evaluate their difference, using concentrations and reagents presented in table 2.2.

Table 2.2. Reagents and Concentrations of aqueous solutions for the second tasting.

Sensations/ Feeling	Concentration and Reagent
Sour + Sweet	1 g/L Tartaric Acid + 10g/L Sucrose
Sour + Astringency	1 g/L Tartaric Acid + 0,8g/L Aluminium Sulphate
Sour + Sweet + Hot	1 g/L Tartaric Acid + 10g/L Sucrose + 5% Ethanol
Sour + Sweet + Hot + Astringency	1 g/L Tartaric Acid + 10g/L Sucrose + 5% Ethanol + 1 g/L Skin Tannin
Body	2 g/L Soft Gum
Body	0.2 g/L Carboxymethylcellulose
Body	0.6 g/L Mannoprotein

Soft Gum was purchased from A. Freitas Vilar, Lda (Lisboa, Portugal), Carboxymethylcellulose (Cistab Gc) was purchased from Proenol (Porto, Portugal), and Mannoproteins were purchased from Laffort (Bordeaux, France).

In the third tasting, after a week, a triangular test was made with white and red wines from ISA. It was a triangular tasting with two control wines and one glass with an added reagent. Participants had to detect and recognize the different sample. The wine based solutions were made with reagents presented in table 2.3. The following tasting (fourth tasting), after a week, was made in the same way because it was noticed a lack of concentration and a difficulty concerning the participants. Tannic Acid was purchase from M&B (Dagenham, England).

Table 2.3. Reagents and Concentrations for the third and fourth tasting

Wine	Sensations/ Feeling	Concentration	Reagent
White	Sweet	30 g/L	Sucrose
White	Sour	2 g/L	Tartaric Acid
Red	Bitter	15 mg/L	Quinine Sulphate
Red	Astringency	1 g/L	Tannic Acid



The fifth tasting was the last session before the final panel selection. It was a triangular tasting as the third and fourth tastings but with lower concentrations to hamper the tasting. The concentrations and reagents are presented in table 2.4.

Table 2.4. Reagents and Concentrations for the fifth tasting.

Wine	Sensations/ Feeling	Concentration	Reagent
White	Sweet	20 g/L	Saccharose
White	Sour	0.66 g/L	Tartaric Acid
Red	Bitter	5 mg/L	Quinine Sulphate
Red	Astringency	0.5 g/L	Tannic Acid

For selecting the final panel it was considered the results from the last tasting. Participants had to have at least 75% of the correct answers.

### 2.1.2. Selected Panel

The selection began with 41 students and finished with 21 people, with 7 females and 14 males, age 21-46 years (average 25 years, standard deviation 5.79). There were nine students from the first year of the Viticulture and Oenology Master but also non-enology students. All the participants were asked about their background in terms of studies for since how long they drink wine, how many times per week do they drink wine and what they consider themselves about drinking wine (expert, interested or drink for enjoyment).

### 2.1.3. Sensory Astringency session

Panelists were familiarized with samples and tasting procedures in five hour training sessions. They had been introduced first to the theory of astringency and extensively trained to differentiate astringency from bitterness and sourness using 1.5 g/L tannic acid, 15 mg/L quinine sulphate and 1.5 g/L tartaric acid as examples of astringency, bitterness and sourness, respectively. They were asked to evaluate overall astringency of different concentrations (from 0.1 to 5.0 g/L) of CT (commercial tannin extracted from grape seeds, Tanipepin, Proenol, Porto, Portugal) on a 9-point scale (named: absent, very weak, weak, weak moderate, moderate, moderate strong, strong, very strong, extremely strong) first in water and then in white wine. At the beginning of each session, panelists tasted the standard reference solutions for astringency, which consisted of three CT wine solutions (very weak astringency = 0.1 g/L; moderate astringency = 2.5 g/L; extremely strong astringency = 5.0 g/L) representing the intensities of the sensation on the 9-point scale. During the tasting sessions, four samples (CT wine solutions or wines) were evaluated in duplicate. In each session samples (10 mL) were presented in balanced random order at room temperature ( $18 \pm 2$  °C) in black tulip-shaped glasses coded with 3-digit random numbers. The assessors were instructed to pour the whole sample in their mouth, hold it for 8 s, expectorate and rate the



perceived overall astringency using a 9-point scale. Judges waited for 4 min before to rinse with de-ionised water for 10 s twice and then waited at least 30 s before the next sample (each sample was evaluated within 5 min) (Rinaldi 2012).

## **2.2. Taster characterization**

Tasters were classified by Vinotype result, online test ([www.vinotype.com](http://www.vinotype.com)) based on the individual's wine preferences (Hanni, 2012), Saliva mean flow, measured according to the procedure described by Smith et al (1996) and with PROP status through the bitterness intensity of three PROP (Sigma, St. Louis, USA) solutions (0.032, 0.32 and 3.2 mM) that were present in increasing order of concentration.

PROP status was assessed during two fifteen minutes sessions held over 2 to 3 weeks. Participants rated the bitterness intensity of three PROP (Sigma, St. Louis, USA) solutions (0.032, 0.32 and 3.2 mM) that were present in increasing order of concentration. Individuals were given 20 ml of solution in each glass and instructed to move the solution in the mouth and to gargle for 5 to 10 seconds and to expel the sample (Pickering et al., 2004). After 10 to 15 seconds, they used a gVAS scale (generalized Visual Analog Scale) to rate the bitterness of the samples. According to Pickering et al. (2016), the gVAS scale uses a “no sensation” on the left (0 mm) and on the right “the worst sensation imaginable” (100 mm). After researching the gVAS scale it was noticed that each author uses a different mark on the scale, some use no mark at all (Hayes, 2013) and others at 25, 50 and 75 mm (Pickering et al., 2016). It was decided to use only the 50 mm mark just to have a reference.

Participants were classified as non-tasters, tasters or super-tasters based in the bitterness rating assigned to the 0.32 mM PROP solution using the gVAS scale (non-taster:  $\leq 15.5$ ; taster:  $> 15.5$  and  $< 51$ ; super-taster:  $\geq 51$ ) (Tepper et al., 2001).

## **2.3. Detection and Recognition best estimate thresholds**

This method was applied to find out two different thresholds: the Detection and the Recognition Threshold. The Detection Threshold identifies at which concentration the subject is able to distinguish a sample from the other. The Recognition Threshold identifies at which concentration the subject is able to identify which kind of difference. The BET is applied to the answers of every single subject and then calculated with the final average of the panel. In both cases, the BET is the tool through which is possible to calculate and relate the physical intensity of the stimulus to the corresponding sensation and converting to a value (Meilgaard et al., 1999).

The determination of taste Detection and Recognition Thresholds was done by the selected tasting panel using 4 different concentrations of CT (Tanipepin, Proenol, Porto, Portugal), added to an Arinto base wine presented in balanced random order at room temperature ( $18 \pm 2$  °C) provided with 30 mL of wine in coded standard clear wine glasses (ISO-3591, 1997) in black tulip-shaped .

The panelists each received six 3-Alternative Forced Choice (3-AFC) tests with ascending concentrations, starting from 0.02 to 0.32 g/L in wine. In each 3- AFC presentation, three samples are presented: two were controls, and one contained the substance under test. The judges examined each sample from left to right and selected the odd wine. Randomization of the position of the different sample, within each 3-AFC presentation, was carried out for the different panelists; the option of going back to repeat the evaluation of each sample was possible within the single 3-AFC presentation but not possible among the different 3-AFC groups of wines. The best estimate threshold (BET) for each subject was evaluated as the geometric mean of first correct answer of all higher concentrations correct and last incorrect step.

## **2.4. Determination of astringency, persistency and preference**

### **2.4.1. Scale utilization**

Subjects were trained in the use of the general labeled magnitude scale (gLMS) following published standard procedures (Bartoshuk 2000; Green et al. 1993, 1996) that involved culturally appropriate remembered or imagined sensations (Annex 1). The gLMS is a psychophysical tool that yields high quality, ratio level data (Bartoshuk 2000). It requires subjects to rate their perceived intensity of a given stimulus along a line scale with adjectives at empirically derived intervals. The 100 point scale comprises the following adjectives: no sensation= 0, barely detectable=1.5, weak=6, moderate=17, strong=35, very strong=52, and the strongest imaginable sensation of any kind=100 (Bartoshuk 2000). The scale presented to subjects shows only the adjectives, not the corresponding numbers. The score, in cm, for each of the intensity measures was manually obtained with a ruler.

To control for idiosyncratic scale usage, subjects were asked to rate the heaviness of six visually identical weights (sandfilled opaque jars of weights 53, 251, 499, 724, 897, and 1127 g). Subjects held out their dominant hand, palm up while the experimenter placed the weighted bottle on the palm of the hand. Subjects were instructed to use the gLMS to rate the heaviness of each weight.

The scale for preference was a gDOL scale, using an individual generalized degree of liking (gDOL) scales, anchored with three adjectives: “Strongest Disliking of any kind” at the bottom of the scale, “Strongest Liking of any kind” at the top, and “Neutral” in the middle. (Byrnes et al., 2013).

### **2.4.2. Wines**

The wines were purchased from local outlets and were selected among several Portuguese wine regions in order to obtain samples eliciting different perceptions of astringency (Table 2.5).

One sample was a control wine (WC) composed by Arinto base wine (ISA) spiked with supra-threshold concentrations of CT (1.5 g/L) and tartaric acid (1.2 g/L).

Table 2.5 Wines used in the study.

Wines	Brand	Region	Notes
<b>W1</b>	Piteira DOC Reserva 2012	Alentejo	Bland of typical regional varieties.
<b>W2</b>	Marquês de Marialva DOC Reserva 2010	Bairrada	Baga 100%, aging for 9 months in French oak barrels.
<b>W3</b>	Quinta das Bageiras Colheita Tinto 2012	Bairrada	Baga 100%, end fermentation and aging in old oak barrels for 18 months.
<b>W4</b>	Casas Altas DOC Reserva 2011	Beira Interior	Touriga Nacional 100%, fermentation in stainless steel and aging for 12 months in French oak staves.
<b>W5</b>	Aguião IGT 2014	Minho	Vinhão and Borraçal, fermentation and storage in stainless steel.

## 2.5. Statistical analysis

Homoscedasticity of the samples was assessed by Levene's test prior to variance analyses (ANOVA). As all the samples showed homogeneity in the variances, they were subjected to the different ANOVAs. When the ANOVAs revealed significant differences between levels, Tukey's test (in the case of more balanced sets) or LSD's test (in the case of non-balanced sets of samples) were performed using Microsoft Excel and Statistix 9.0 software, with  $\alpha=0.05$ .

### 3. Results and discussion

#### 3.1. Taster Function

The evaluation of the taster phenotype was performed using the responses to increasing concentrations of PROP. The results were the average of two measurements, revealing the expected 3 classes of sensitivity (non-tasters, tasters and super-tasters) as reported by Pickering *et al.* (2004). These authors used the bitterness rating assigned to the 0.32 mM PROP solution as the indicator to distinguish between the 3 classes (non-taster  $\leq 15.5$ ;  $15.5 < \text{taster} < 51$ ; super-taster  $\geq 51$  mm). Accordingly, in our study the individuals were considered mostly tasters (8) and supertasters (10) while there were only 3 non-tasters. The results of the PROP phenotype for each of the tasters have already been presented in the work of Cecilliani (2017).

The responses to the Vinotype questionnaire yielded, on a total of 21 tasters, 4 Hypersensitive, 12 Sensitive and 5 Tolerant tasters. The amount of saliva produced by the tasters (average of 2 determinations) was already published by Cecilliani (2017). The lowest value was 1.416 g/min and the highest 4.466 g/min with a total average of 3.2 g/min, with a regular increase in the amount produced within the range.

The overall segmentation of the tasters is shown in Table 3.1. Regarding gender, we observed that males and females were distributed among the other 3 segments. The low number of respondents does not allow to draw definite conclusions but it is interesting to note that all low Saliva producers were PROP non-tasters. In addition, there were no Vinotype hypersensitives among the PROP non-tasters.

Table 3.1. Taster characterization according to the segment.

		Gender		Vinotype			Saliva		PROP		
		M	F	T	S	HS	H	L	NT	T	ST
Gender	M	<b>14</b>	-	3	9	2	5	9	2	7	5
	F	-	<b>7</b>	2	3	2	2	5	1	1	5
Vinotype	T	3	2	<b>5</b>	-	-	2	2	1	1	3
	S	9	3	-	<b>12</b>	-	4	8	2	6	4
	HS	2	2	-	-	<b>4</b>	1	3	0	1	3
Saliva	H	5	2	2	4	1	<b>8</b>	-	0	1	6
	L	9	5	2	8	3	-	<b>13</b>	3	7	4
PROP	NT	2	1	1	2	0	0	3	<b>3</b>	-	-
	T	7	1	1	6	1	1	7	-	<b>8</b>	-
	ST	5	5	3	4	3	6	4	-	-	<b>10</b>

Gender= M-male; F-female

Vinotype= T-tolerant; S-sensitive; HS-hipersensitive

Saliva= H-high flow; L-low flow

PROP= NT-non taster; T-taster; ST-supertaster

### 3.2. Sensory thresholds of commercial grape seed tannin

The first set of trials was done with 0.2 to 1.6 g/L tannin. The concentrations detected as different from the blank or recognized as more astringent are shown in Table 3.2. These results enabled the calculation of the Best Estimate Threshold (BET) for the detection and for the recognition. However, when we plotted these results (Figure 3.2) we could not see a clear tendency to increase in the percentage of individuals detecting the tastant. In addition, the BET calculation through the graph (Figure 3.2) gave a value of about 0.26 g/L which very different from the value of 0.64 g/L shown in Table 3.2. Therefore we did another trial with higher concentrations of tannin. The results shown in Table 3.3 enabled to calculate the BET for detection as 0.95 g/L. In addition, Figure 3.3 shows that the panel clearly recognized the different increasing concentrations of tannin. In this case, the BET was visually calculated by interpolation giving a result of 0.80 g/L.

Table 3.2. Best estimated threshold (BET) calculation for the Detection and Recognition thresholds of commercial tannin (g/L). Correct choice indicated by 1 and incorrect by 0; highlighted gray cells indicate recognition of astringent taste.

Taster	0.2	0.4	0.8	1.6	Detection threshold		Recognition threshold	
1	1	1	1	1	0.07	-1.15	0.57	0.05
2	0	1	1	1	0.14	-0.85	1.13	0.66
3	1	1	0	1	0.57	-0.25	1.13	0.66
4	1	0	0	0	1.13	0.05	1.13	0.66
5	1	1	1	1	0.07	-1.15	0.07	0.66
6	1	1	1	0	1.13	0.05	1.13	0.66
7	0	1	0	0	1.13	0.05	1.13	-0.25
8	0	1	1	1	0.14	-0.85	1.13	-0.25
9	0	1	1	1	0.14	-0.85	0.14	0.66
10	1	1	1	0	1.13	0.05	1.13	0.66
11	0	0	1	0	1.13	0.05	1.13	0.05
12	1	0	0	0	1.13	0.05	1.13	-0.70
13	1	0	1	1	0.14	-0.85	0.57	0.66
14	0	1	1	1	0.14	-0.85	1.13	0.66
15	1	1	0	1	0.57	-0.25	1.13	-0.25
16	0	0	1	1	1.13	0.05	0.57	0.66
17	1	1	0	1	0.57	-0.25	0.57	-0.25
18	0	1	1	1	0.14	-0.85	0.57	-0.25
19	0	0	1	1	0.28	-0.54	0.28	0.66
20	1	1	0	1	0.57	-0.25	0.57	0.66
21	0	1	1	1	0.14	-0.85	1.13	-0.25
					Log BET	Antilog	Log BET	Antilog
					-0.45	0.64 g/L	-0.15	0.86 g/L

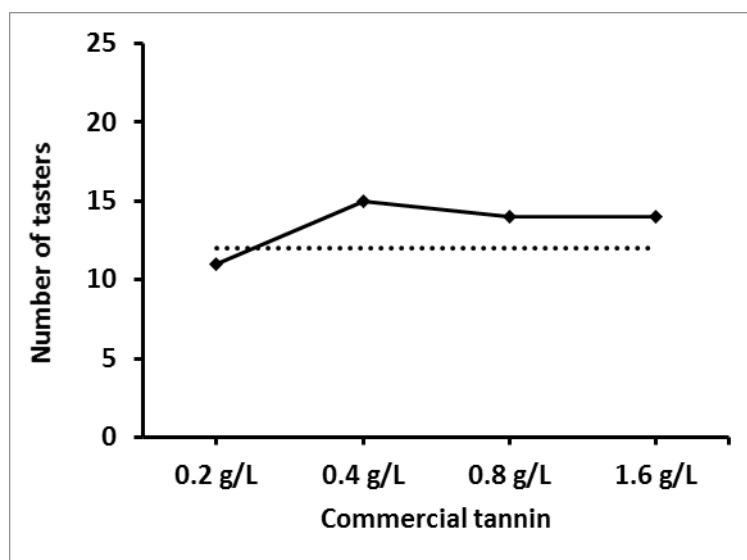


Figure 3.2. Geometric trend of Detection Threshold of Commercial Tannin. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line ( $n = 12$ ) represents minimum agreeing judgments necessary to establish preference using  $\alpha=0.05$  for triangular comparison tests (total number of tasters  $N=21$ ).

Table 3.3. Best estimated threshold (BET) calculation for the Detection and Recognition thresholds of commercial tannin (g/L). Correct choice indicated by 1 and incorrect by 0; highlighted gray cells indicate recognition of astringent taste.

Taster	0.4	0.8	1.6	3.2	Detection threshold		Recognition threshold	
1	1	1	1	1	0.28	-0.54	2.26	0.35
2	0	1	1	1	0.57	-0.25	2.26	0.35
3	0	1	1	1	0.57	-0.25	0.57	-0.24
4	0	0	1	1	1.13	0.05	1.13	0.05
5	1	1	1	1	0.28	-0.54	0.28	-0.54
6	1	1	1	1	0.28	-0.54	0.28	-0.54
7	0	1	1	1	0.57	-0.25	2.26	0.35
8	0	0	0	1	2.26	0.35	2.26	0.35
9	0	1	1	1	0.57	-0.24	0.57	-0.24
10	0	0	0	1	2.26	0.35	2.26	0.35
11	0	0	0	1	2.26	0.35	4.52	0.65
12	0	0	1	1	1.13	0.05	1.13	0.05
13	0	0	1	1	1.13	0.05	1.13	0.05
14	1	1	1	1	0.28	-0.54	1.13	0.05
15	1	1	0	0	2.26	0.35	4.52	-0.65
16	1	1	1	1	0.28	-0.54	4.52	0.65
17	1	0	1	1	1.13	0.05	2.26	0.35
18	0	1	1	1	0.57	-0.25	2.26	0.35
19	0	0	1	1	1.13	0.05	1.13	0.05
20	0	0	1	1	1.13	0.05	2.26	0.35
21	0	0	1	1	1.13	0.05	1.13	0.05
					<b>Log BET</b>	<b>Antilog</b>	<b>Log BET</b>	<b>Antilog</b>
					-0.05	0.95 g/L	0.19	1.21 g/L

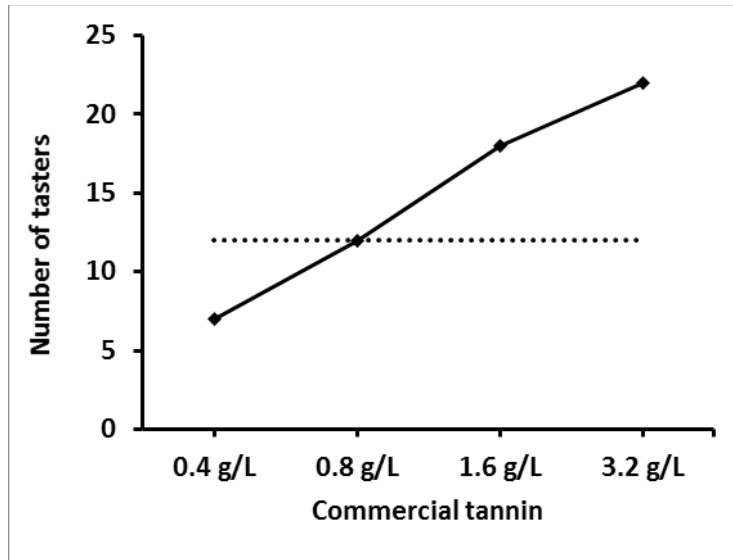


Figure 3.3. Geometric trend of Detection Threshold of Commercial Tannin. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line ( $n = 12$ ) represents minimum agreeing judgments necessary to establish preference using  $\alpha=0.05$  for triangular comparison tests (total number of tasters  $N=21$ ).

The BET for Recognition could not be achieved with the lower concentrations (Figure 3.4). The higher concentrations of tannin enabled to establish a recognition BET of 1.33 g/L (Figure 3.5) which is similar to the value of 1.21 g/L reported in Table 3.3.

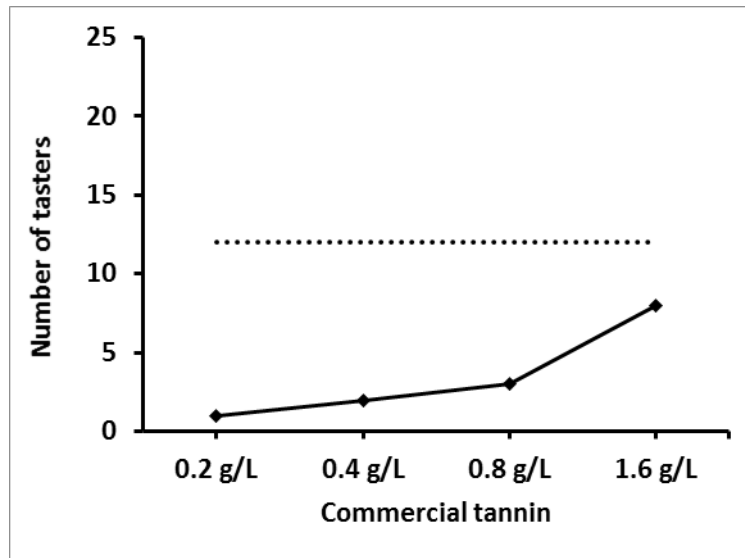


Figure 3.4 Geometric trend of Detection Threshold of Commercial Tannin. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line ( $n = 12$ ) represents minimum agreeing judgments necessary to establish preference using  $\alpha=0.05$  for triangular comparison tests (total number of tasters  $N=21$ ).

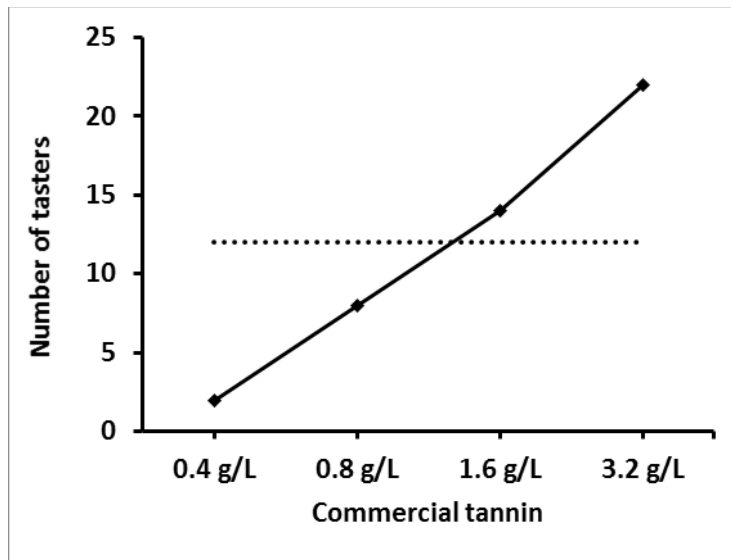


Figure 3.5. Geometric trend of Detection Threshold of Commercial Tannin. Number of tasters (♦) able to detect the respective added sample at each concentration. Dotted line ( $n = 12$ ) represents minimum agreeing judgments necessary to establish preference using  $\alpha=0.05$  for triangular comparison tests (total number of tasters  $N=21$ ).

### 3.3. Relation among the taste functions

#### 3.3.1. Relation between PROP response and Saliva flow

Figure 3.6 shows the correlation between the saliva flow and PROP intensity measure for all 21 tasters. Despite being a positive correlation the value of  $R$  was only 0,34 ( $p=0.129$ ) and so we could not establish a significant correlation between PROP response and salivary flow for the tasting panel.

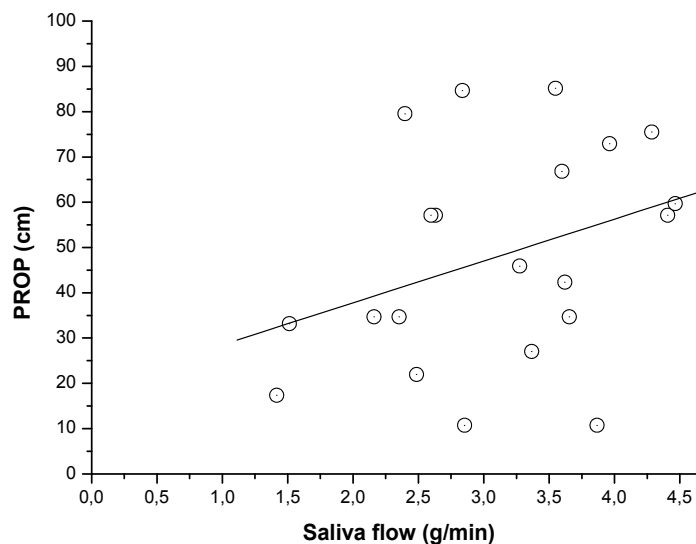


Figure 3.6. Individual responses to PROP and saliva flow for the tasting panel.



### 3.3.2. Relation between detection and recognition threshold

The values of BET reported earlier may be used to check if there is a relation between detection and recognition of tannin. The Figure 3.7 shows that although positively correlated these two functions had a poor R (0.46,  $p=0.034$ ). Therefore, it seems that the ability to detect tannin is independent of the ability to identify it.

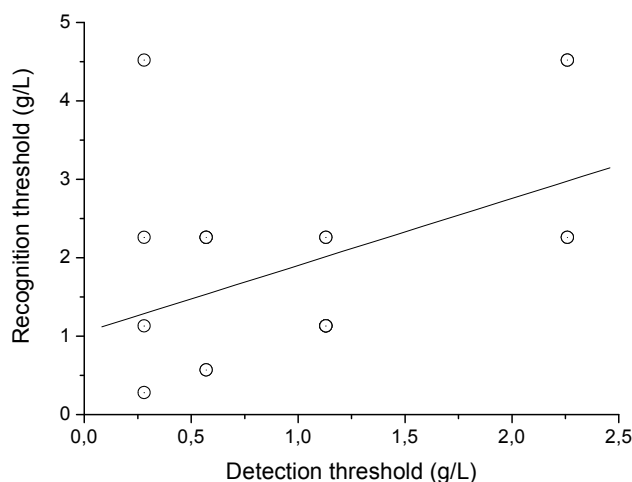


Figure 3.7. Relation between the detection and recognition thresholds for the 21 tasters (symbols overlap and do not show all responses).

### 3.3.3. Relations between sensory thresholds, PROP responses and saliva flow

The previous correlations were also tried concerning sensory thresholds and PROP intensity and saliva flow. We observed poor negative correlations ( $r<0.5$ ) among the two sensory thresholds and PROP or saliva flow (results not shown). Therefore, although tendencies to have higher sensitivities to tannin (lower BET's) with higher PROP sensitivity or saliva flow was observed, we could not find a significant correlation.

As a consequence of the previous results, we should evaluate the responses to wine astringency according to PROP status and Saliva flow.

### 3.4. Evaluation of astringency, persistency and preference in commercial red wines

The sensory panel evaluated the astringency and persistence of five different red wines. When scores were pooled together for all wines we observed a positive relation between astringency and persistency ( $r= 0,71$ ,  $p<0.0001$ ) (Figures 3.8). However, we found no relation between these mouthfeel attributes and the preference indicating that other sensory characteristics influence wine liking, the results are not shown.

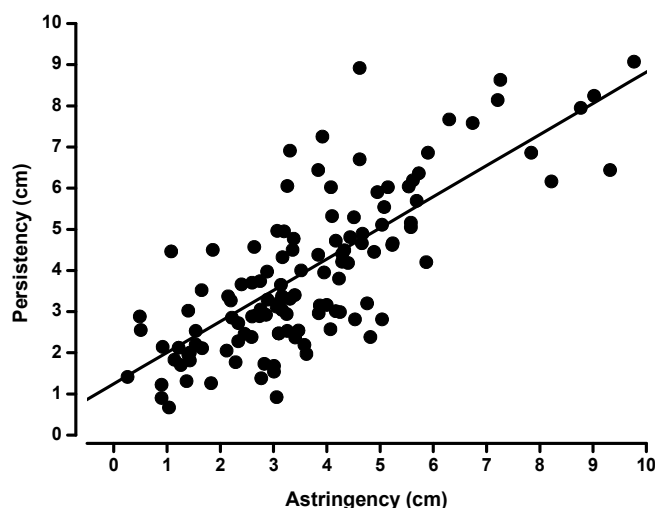


Figure 3.8. Relation between the astringency and persistency of commercial red wines.

ANOVAs results are showed in table 3.4. It is possible to observe as WC (wine control) displayed a higher intensity astringency and persistency rate (similar with the W3). The W5, young wine from Minho, north region of Portugal, showed a low astringency and a high persistency, maybe according to the freshness and acidity of the wine. Wines W1, from Alentejo, showed a lower intensity rate of persistency then the other wines under trials. Is interesting observe the tendencies by the W3 and W4, from north of Portugal, both evaluated with a higher astringency and persistency rate. The wine W2, from the Bairrada as the W3, showed different results maybe for the different vinification and aging process. To better compare the different mean evaluations of the wines under study, the results are showed in Figure 3.9.

Table 3.4 Tuckey's test of wines under study for astringency, persistency and preference intensities.

Descriptor	W1	W2	W3	W4	W5	WC
Astringency	3.38 bc	3.07 c	4.58 a	4.35 ab	2.77 c	4.63 a
Percistency	3.32 c	3.86 abc	4.69 ab	4.60 abc	3.70 bc	5.27 a
Preference	10.78 a	11.04 a	10.65 a	9.97 a	10.61 a	9.75 a

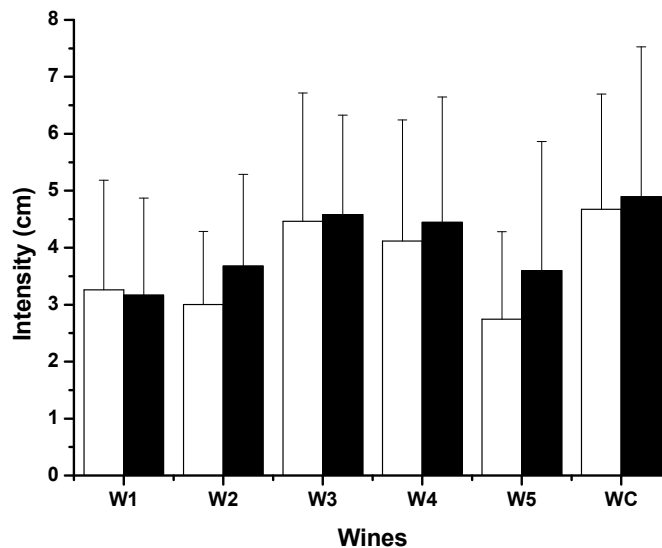


Figure 3.9. Intensity of astringency (white bar) and persistency (black bar) of each tasted wine.

### 3.5. Taster responses according to segmentation

The previous results were obtained considering together the responses of all individuals for the five commercial wines. It would be interesting to check if we could find any relation between the taste functions of each taster and the overall mean responses of the astringency and persistency for the five wines, including the responses to the control wine. It should be noted that some segments have a low number of individuals (see Table 3.1). Levene's test showed that homoscedasticity of the samples could be generally assumed. The ANOVAs revealed significant differences between levels, Tukey's test (in the case of more balanced sets) or LSD's test (in the case of non-balanced sets of samples) showed significant differences in the Gender and Saliva flow.

#### 3.5.1. PROP status

The Figure 3.10. shows the PROP status responses according to the different intensity of all wines under trials. We did not find significant difference regarding the response to the intensities under study but a slightly trend for non-taster status to rate the astringency intensity with lower sensitivity than taster and super-taster status, was observed. As showed in the table 3.1, only three subjects were characterized as non-taster.

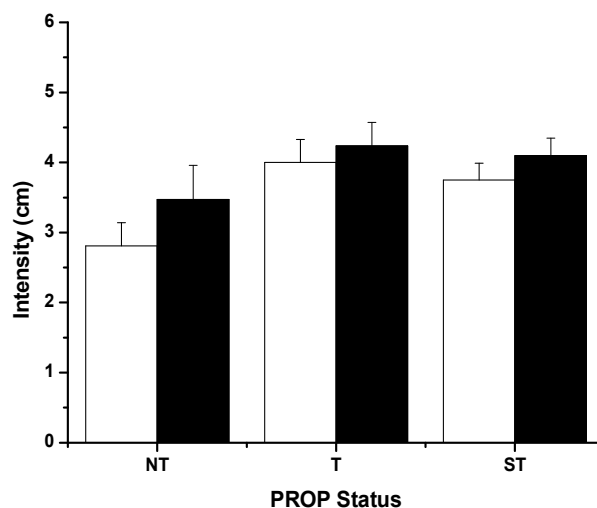


Figure 3.10. Intensity of astringency (white bar) and persistency (black bar) of all tasted wines according to PROP status (NT, Non-taster, T, Taster, ST, Supertaster). Horizontal bars indicate standard error.

### 3.5.2. Vinotype

As show the figure 3.11, we could not find any relation between the Vinotype and the response to the intensity valuation for the astringency and persistency. The number of tasters for each class, does not permit to go further with reliable statistical analysis.

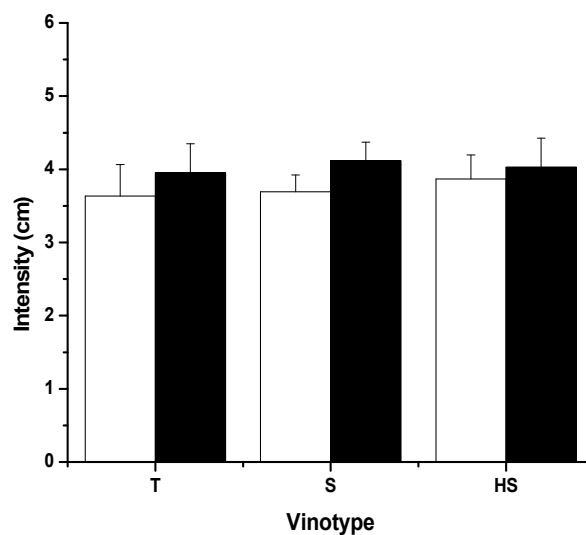


Figure 3.11. Intensity of astringency (open bar) and persistency (black bar) of all tasted wines according to Vinotype (T, Tolerant; S, Sensitive; HS, Hypersensitive). Horizontal bars indicate standard error.

### 3.5.3. Saliva flow

A significant difference was found between the Saliva low and high flow, according to the astringency and persistency intensity, as showed in the Figure 3.12.

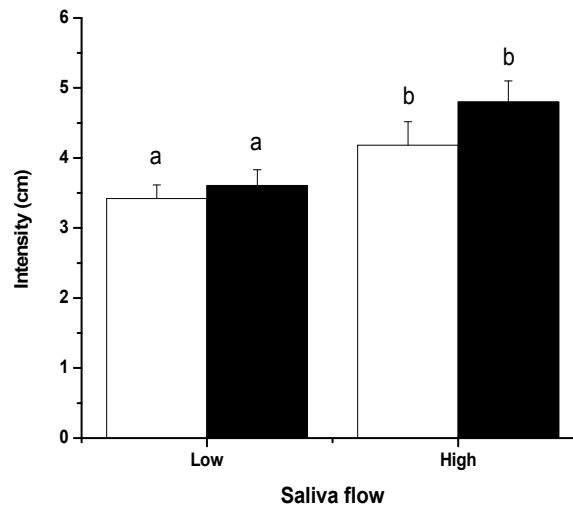


Figure 3.12. Intensity of astringency (open bar) and persistency (black bar) of all tasted wines according to Saliva flow (L, low; H, high). Different letters for the same attribute indicate significant differences ( $p < 0.05$ ). Horizontal bars indicate standard error.

Tuckey's test revealed differences among the taster segmentation, as shown in table 3.5. Saliva high flow tasters have a higher rate in astringency and persistency intensities compared to Saliva low flow tasters.

Table 3.5. Tuckey's test between Saliva flow and the astringency and persistency intensities.

Descriptor	Saliva Low flow	Saliva High flow
Astringency	3.41 a	4.20 b
Percistency	3.60 a	4.80 b

### 3.5.4. Gender

As show figure 3.13, differences among the Gender segmentation according to the intensity valuation for the astringency and persistency was found.

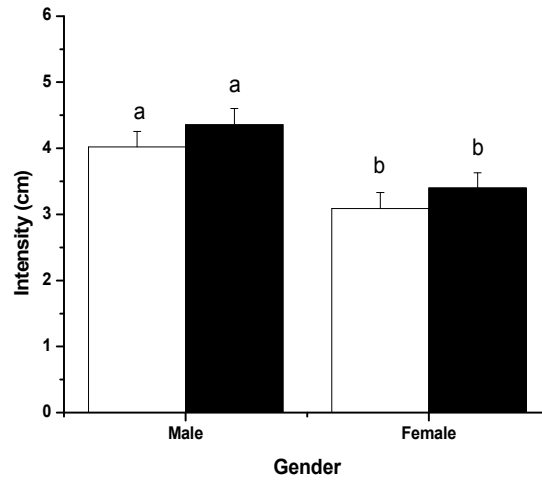


Figure 3.13. Intensity of astringency (open bar) and persistency (black bar) of all tasted wines according to Gender (M, Male; F, female). Different letters for the same attribute indicate significant differences ( $p < 0.05$ ). Horizontal bars indicate standard error.

LSD test revealed a significant difference of males to detect higher astringency and persistency intensities than the females, as shown in the table 3.6.

Table 3.6. LSD test between gender and astringency and persistence intensities.

Descriptor	Female	Male
Astringency	3.15 b	4.10 a
Persistency	3.47 b	4.35 a

### 3.6. Wine evaluation according to segmentation

#### 3.6.1. Saliva flow

The last part of the study was to observe differences within the segmentation according to the intensities descriptors. For each wine, astringency and persistency mean rates of low and high salivators, and of male and female were also compared using T-Student's test for independent samples, with a significance level of 0.05. As shown in table 3.7, there was a different evaluation among Saliva low flow and high flow according to the astringency intensity. It is less noticeable considering the W5 and WC, respectively the lowest and highest astringent wines (see table 3.4), where the results seems to give a parameter more homogeneous. On the contrary considering persistency intensity, the difference between high and low saliva flow was higher for all wines, as had been shown in table 3.5. The single wines showing statistical difference were W2 and W4 for the persistency and astringency, respectively (see annex 8 and 9).

Table 3.7 Mean evaluation of wines for astringency and persistency according to Saliva flow (significant differences in bold), T-Student's test.

Descriptor	W1		W2		W3		W4		W5		WC	
	L	H	L	H	L	H	L	H	L	H	L	H
Astringency	2.91	3.94	2.82	3.41	4.14	5.11	<b>3.47</b>	<b>5.12</b>	2.70	2.92	4.53	4.82
Persistency	2.67	3.97	<b>3.06</b>	<b>4.66</b>	4.21	5.17	4.02	5.16	3.22	4.18	4.44	5.61

#### 3.6.2. Gender

The Table 3.8 showed a different trend in particular regarding the WC, where Females rated it as the third most astringent and second most persistent wine, while Males valued it as the most astringent and persistent wine of all. The single wine showing statistical differences was W2 regarding astringency (see annex 6 and 7), while no differences for persistency was observed.

Considering the mean evaluation for all wine, we can confirm that the panel distinguished and recognized at the same way but with a different sensibility the wines under study, where the WC displayed with the W3 and W4 as the most astringent and persistence.

Table 3.8 Mean evaluation of wines for astringency and persistency according to Gender (significant differences in bold), T-Student's test.

Descriptor	W1		W2		W3		W4		W5		WC	
	F	M	F	M	F	M	F	M	F	M	F	M
Astringency	2.21	3.78	<b>2.22</b>	<b>3.39</b>	4.13	4.62	4.07	4.16	2.23	3.10	3.75	5.13
Persistency	2.54	3.47	3.12	3.94	4.16	4.78	3.90	4.73	3.05	3.88	4.09	5.29

#### 4. Discussion

In this work we established the Detection and Recognition Thresholds of commercial tannins extracted from the grape seeds, added in Arinto wine from the ISA. The first set of trials gave a value of about 0.26 g/L, in addition, the second set with higher concentrations enabled to calculate the BET for detection as 0.95 g/L and for recognition as 1.21 g/L. The correlation between detection and recognition of tannin was poor ( $r=0.45$ ). Therefore, it seems that the ability to detect tannin is independent of the ability to identify it.

In the sensory evaluation of the five Portuguese red wines, we observed a positive relation between astringency and persistency ( $r= 0.71$ ,  $p<0.0001$ ). In addition, the aim was to check any relation between the taste functions of each taster and the overall mean responses of the astringency, persistency and preference. We could not find a strong relation between PROP response and astringency ( $r=0.38$ ), although a better correlation was found with persistency ( $r=0.46$ ). The same tendency was observed for the relation between the Astringency ( $r=0.26$ ) and Persistency ( $r=0.43$ ) with the Saliva flow response.

Ishikawa and Noble (1995) found no relationship between PROP taster status and the perception of astringency in Carnelian red wine. Pickering et al. (2004) founded a relation between taster sensitivity and astringency perception. We observed that non-taster were less sensitive to astringency sensation than tasters and super-tasters status in some wines, but we did not find a significant relationship.

The last sections dealt with the evaluation of the scores given to each wine, including the Arinto wine control added concentrations of CT (1.5 g/L) and tartaric acid (1.2 g/L). Considering the single taster of the selected panel, and subsequently sharing it for the taste function according to the different phenotype, we observed two different tendencies regarding the astringency and persistence response, respectively for the W1, W2 and W5, that showed a lower intensity rate, and the W3, W4 and WC, apparently with a higher intensity rate. These results are in agreement with the regions and styles of the wines. The lower perceptions of astringency were for Alentejo, Bairrada and Minho. This Bairrada was not a typical one because due to the oak aging and high ethanol level it looked as if was a smooth wine. The wines W3 and W4 were typical examples of Bairrada and Beira Interior and were grouped with the white wine spiked with tannins and tartaric acid. These results should have been compared to the chemical analysis of the wines, especially those parameters related with astringency like gelatin Index (Glories 1984) and Tannic Power (Freitas and Mateus, 2001).

In spite of the before mentioned differences due to astringency, it was interesting to see that preference was similar for all wines. Therefore, the different astringency levels were not different enough to express different preferences. However we may also hypothesize that our group of highly trained tasters, focused more attention on the different wine styles in order to response the intensities perception than the wine preference.



## 5. Conclusion and future prospects

In this work the aim was the segmentation of a panel test in function to the taste phenotype characterization, to investigate the response to astringency in five different Portuguese red wines. The results obtained showed a subjectivity to detection and recognition of astringency, considering the main difficult to found a common vocabulary to describe this sensation in the mouth.

The relation among the astringency and persistency intensity indicates a good relation. Gender and Saliva flow segments had different sensitivity according to the astringency perception. The preference showed no correlation with the ability to detect the astringency, probably more parameters and other factors should be considered in the future.

Astringency is an important sensory characteristic of the wine, considered as a negative attribute if wines were perceived as harsh and rough when drunk alone, but at the same time as a positive and needed attribute if combined with food. Perhaps, even though the results of this study were interesting, a more objective method should be developed in the future, probably combining an astringency chemical indicator, taking into account the multi-perceptual phenomenon of this sensation.

Finally, concerning the tasting panel, we used students that because of continuous training may have a homogenized range of taste sensitivities and unfocused preferences. Thus it would be interesting to include also a wide range of consumers as wine journalists, winemakers and food critics, to better understand the different response and appreciation of astringency in red wines. Therefore, could be interesting investigate this aspect in combination with food pairing, in order to obtain more critical issues and new perspectives adaptable in the wine industry.

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## ANNEX

ANNEX 1. Demographic and physiological characterization of the tasting panel.

<b>Taster</b>	<b>Gender</b>	<b>Age</b>	<b>Vinotype</b>	<b>Saliva (g/min)</b>	<b>PROP Status</b>
<b>1</b>	Female	25	Sensitive	3.96	Super-Taster
<b>2</b>	Female	23	Sensitive	1.42	Taster
<b>3</b>	Male	23	Sensitive	4.29	Super-Taster
<b>4</b>	Male	35	Sensitive	2.35	Taster
<b>5</b>	Male	21	Tolerant	4.41	Super-Taster
<b>6</b>	Male	23	Sensitive	2.68	Taster
<b>7</b>	Male	22	Sensitive	3.37	Taster
<b>8</b>	Male	24	Sensitive	2.16	Taster
<b>9</b>	Female	22	Tolerant	3.6	Super-Taster
<b>10</b>	Male	23	Sensitive	3.45	Taster
<b>11</b>	Male	24	Hipersensitive	3.84	Taster
<b>12</b>	Male	24	Sensitive	1.51	Non-Taster
<b>13</b>	Male	24	Sensitive	3.55	Super-Taster
<b>14</b>	Male	26	Sensitive	4.47	Super-Taster
<b>15</b>	Female	23	Hipersensitive	2.6	Super-Taster
<b>16</b>	Female	28	Tolerant	2.84	Super-Taster
<b>17</b>	Female	22	Hipersensitive	2.4	Super-Taster
<b>18</b>	Male	32	Tolerant	3.27	Non-Taster
<b>19</b>	Female	22	Sensitive	2.85	Non-Taster
<b>20</b>	Male	24	Tolerant	3.65	Taster
<b>21</b>	Male	46	Hipersensitive	2.12	Super-Taster



ANNEX 2- Gender with Astringency ( $\alpha=0,05$ )

	ANOVA	TUKEY	
			MEAN
GENDER	<b>0,0100</b>	MALE	4,0185 <b>A</b>
		FEMALE	3,0952 <b>B</b>

ANNEX 3- Gender with Persistency ( $\alpha=0,05$ )

	ANOVA	TUKEY	
			MEAN
GENDER	<b>0.0262</b>	MALE	4.3509 <b>A</b>
		FEMALE	3.4708 <b>B</b>

ANNEX 4- Saliva flow with Astringency ( $\alpha=0,05$ )

	ANOVA	TUKEY	
			MEAN
SALIVA	<b>0.0267</b>	HIGH	4.1849 <b>A</b>
		LOW	3.4189 <b>B</b>

ANNEX 5- Saliva flow with Persistency ( $\alpha=0,05$ )

	ANOVA	TUKEY	
			MEAN
SALIVA	<b>0.018</b>	HIGH	4.7936 <b>A</b>
		LOW	3.6046 <b>B</b>

ANNEX 6- t-TEST Gender with each wine according to astringency ( $\alpha=0,05$ )

	W1	W2	W3	W4	W5	WC
t-TEST	1.88	2.16	0.46	0.14	1.19	1.52
	0.08	<b>0.04</b>	0.64	0.88	0.28	0.14

ANNEX 7- t-TEST Gender with each wine according to persistency ( $\alpha=0,05$ )

	W1	W2	W3	W4	W5	WC
t-TEST	1.19	1.11	0.75	0.80	0.82	0.99
	0.25	0.28	0.46	0.44	0.41	0.34

ANNEX 8- t-TEST Saliva flow with each wine according to astringency ( $\alpha=0,05$ )

	W1	W2	W3	W4	W5	WC
t-TEST	-1.21	-1.16	-0.98	-2.32	0.32	0.35
	0.23	0.26	0.34	<b>0.03</b>	0.75	0.73

ANNEX 9- t-TEST Saliva flow with each wine according to persistency ( $\alpha=0,05$ )

	W1	W2	W3	W4	W5	WC
t-TEST	-1.78	-2.47	-1.23	-1.16	-0.94	-0.99
	0.10	<b>0.02</b>	0.23	0.26	0.36	0.33

## ANNEX 10. ISA wines analysis

	Free SO <sub>2</sub> (mg/L)	Total SO <sub>2</sub> (mg/L)	pH	Total Acidity (g/L of tartaric acid)	Volatile Acidity (g/L of acetic acid)	Alcohol Strength (%v/v)	Reducing Sugars (g/L)
Arinto 2014	26	90	3.41	5.9	0.24	12	1
Cabernet Sauvignon, Touriga Nacional, Syrah and Trincadeira 2014	36	185	3.76	5.7	0.82	14	1.3

## WINE TASTING – PANEL TEST

## THRESHOLD DETERMINATION

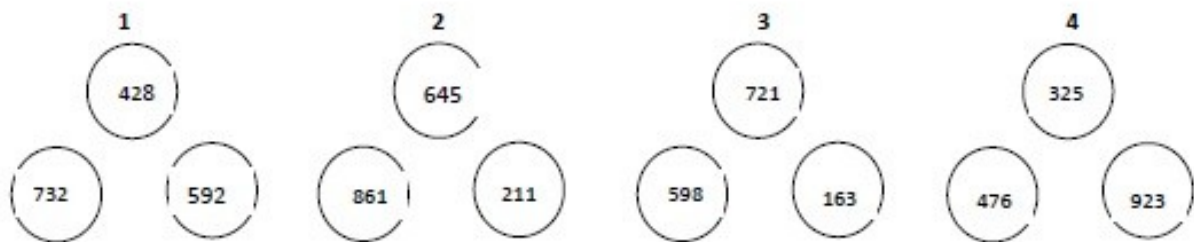
Name: \_\_\_\_\_

Surname \_\_\_\_\_

Age \_\_\_\_\_

Vinotype \_\_\_\_\_

1.1) Taste each set and, inside each one, tell which is the different glass and why. If you are not able to identify the different one, don't leave it white but choose one anyway. After you have finished with one set and move to the next, you cannot come back to the previous ones.



1.2)

	N° of the different one	Reason why is different
1		
2		
3		
4		

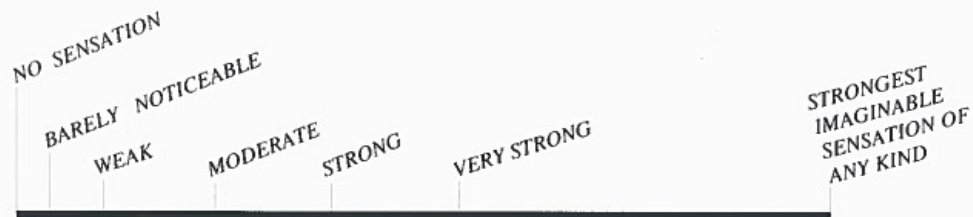
NAME \_\_\_\_\_

SURNAME \_\_\_\_\_

Taste the samples and make your ratings by placing a vertical mark between the descriptors:

**Code samples:**

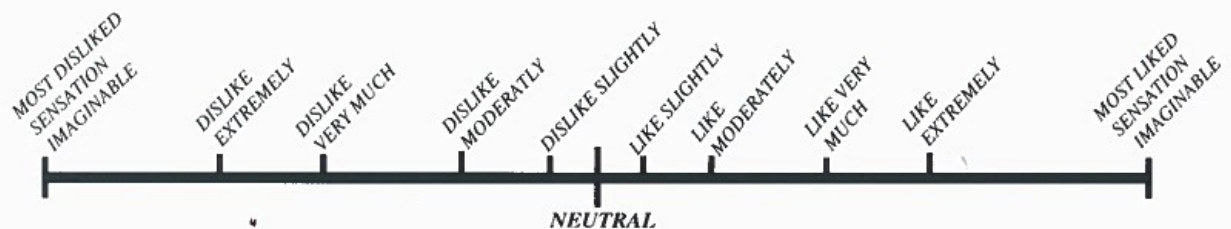
**ASTRINGENCY**



**PERSISTENCE**



**PREFERENCE**



The wine belongs to which Region (select the only one region) :

☐

BAIRRADA

☐

BEIRA INTERIOR

☐

ALENTEJO

☐

MINHO

☐

PALMELA

☐

DOURO